

GENE-ENZYME VARIATION IN THREE SYMPATRIC SPECIES OF *LITTORINA*

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The three littorinid species of the northwestern Atlantic occupy distinct but overlapping habitats in the rocky intertidal zone (Haseman, 1911), from Labrador to the Virginias. The most ubiquitous of these species, *Littorina littorea*, inhabits the middle intertidal, and is believed to be a recent immigrant from western Europe (Sumner, Osborn, and Cole, 1911). This species deposits small egg cases directly into the sea, where development, taking several weeks, proceeds up to the veliger stage. Free swimming larvae then complete their embryonic development in the water column (Purchon, 1968). After some time juvenile adults settle onshore and assume the relatively sedentary life of adult snails. *Littorina obtusata* inhabits the lower intertidal zone subsisting almost entirely off the microflora adhering to either of two algal genera, *Fucus* and *Ascophyllum* (Haseman, 1911). Large gelatinous egg cases of *L. obtusata* are cemented onto the anchored algae, where development proceeds through the larval stages, and the emerging form is a crawling juvenile adult (Purchon, 1968). The third common species, *Littorina saxatilis*, inhabits the upper intertidal and supratidal zones, grazing upon the microflora of encrusted rocks (Haseman, 1911). In contrast to its two sympatric species, *L. saxatilis* has evolved oviviviparity. Eggs are retained within the females' body cavity where development proceeds to the juvenile adult stage (Purchon, 1968).

One possible consequence of these divergent reproductive patterns is immediately apparent. The oviviviparity of *L. saxatilis*, along with its preference for the high intertidal, must severely restrict larval dispersal, and hence limit the potential for interpopulation gene flow. A similar situation may hold for *L. obtusata*, whose eggs and adults face only limited dispersal resulting from the infrequent dislodgement of anchored algae. In contrast, the extensive pelagic period of *L. littorea* eggs and larvae must permit extensive dispersal along the coastal range, in the direction of the predominant longshore ocean currents.

In this study an attempt was made to evaluate the actual consequences of these divergent dispersal capabilities on the distribution of genetic variability in these species, and among their individual populations. The technique of gel electrophoresis was employed, and results from three esterase polymorphisms are discussed.

MATERIALS AND METHODS

Collection sites—and sampling

Fifteen rocky shore localities were sampled, covering a range of about 500 air miles. The localities (Fig. 1) include Charlottetown, Prince Edward Island

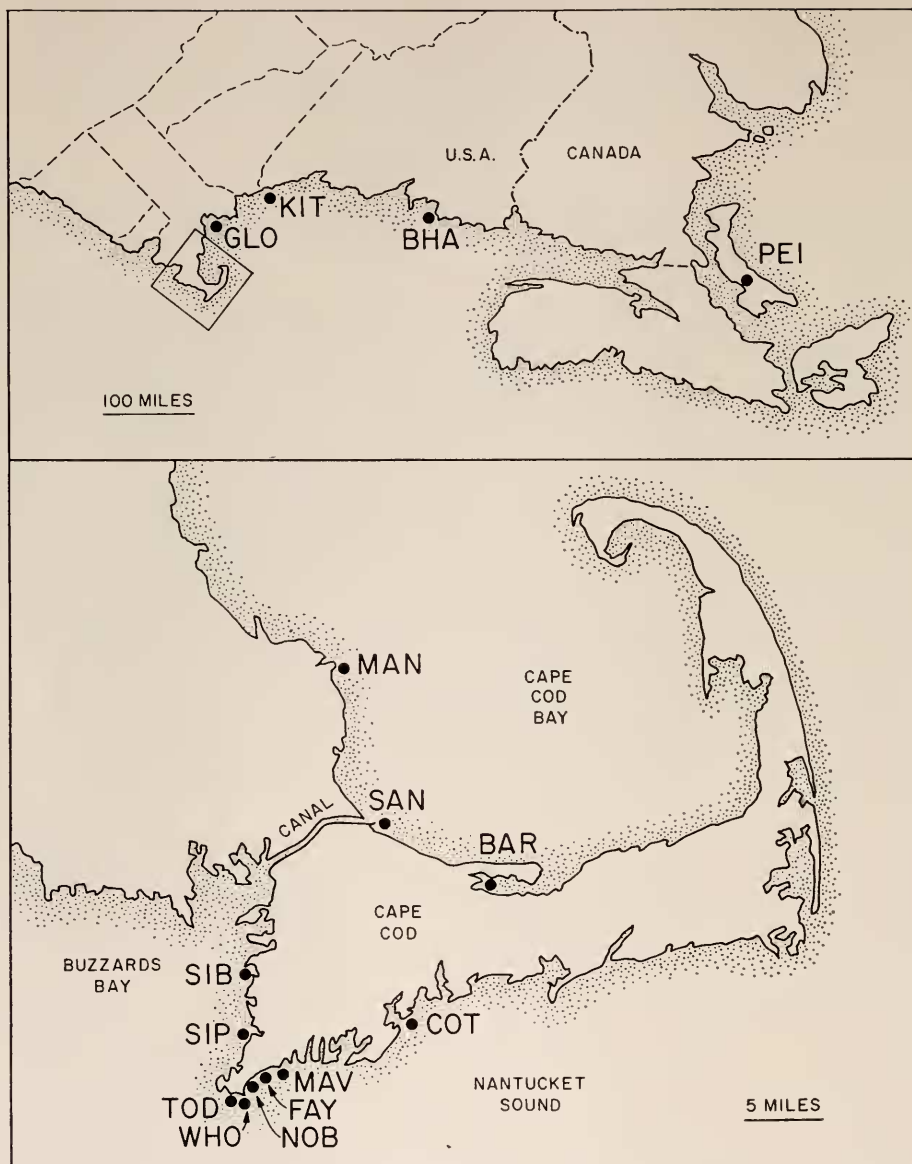


FIGURE 1. Map designations of the 15 collection sites at which *Littorina* populations were sampled. Site designations are explained in Methods.

(PEI); Bar Harbor, Maine (BHA); Kittery, Maine (KIT); Gloucester, Massachusetts (GLO); Manomet Point, Massachusetts (MAN); Sandwich, Massachusetts (SAN); Barnstable, Massachusetts (BAR); Silver Beach, Massachusetts (SIB); Sippewissett, Massachusetts (SIP); the municipal dock (TOD), and fisheries beach (WHO) in Woods Hole, Massachusetts; Nobska Point

(NOB), Fay Beach (FAY), and Mara Vista (MAR) in Falmouth, Massachusetts; and Cotuit, Massachusetts (COT).

L. littorea was collected from 13 of the 15 sites (excluding MAV and FAY); *L. obtusata* from 14 sites (excluding SIB) and *L. saxatilis* from 12 sites (excluding WHO, MAV and COT). Individuals were maintained alive in running sea water until electrophoretic analysis was performed. Between 6 and 43 individuals of each species, from each population, were analyzed electrophoretically for each enzyme system.

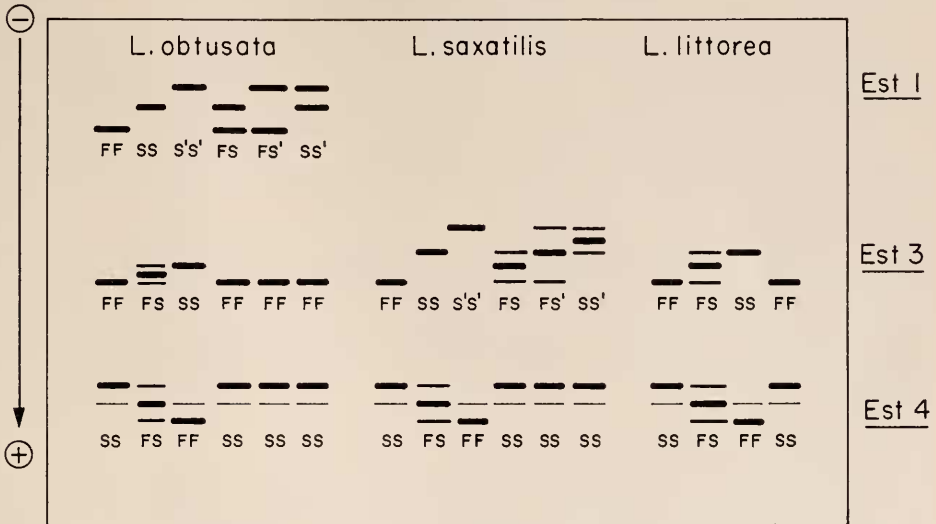


FIGURE 2. Electrophoretic patterns of homozygous and heterozygous genotypes at the three polymorphic esterase loci in the three species of *Littorina*. Enzyme migration in all cases was toward the anode (bottom).

Electrophoresis

Individual snails were removed from their shells and homogenized in two volumes of cold 10% sucrose solution. Homogenates were centrifuged $6000 \times g$ for 10 minutes and aliquots of the supernatant applied directly onto the gels. In many cases the supernatants were stored frozen (-18°C) before analysis. Procedures for acrylamide gel electrophoresis and esterase staining followed the method described by Hubby and Lewontin (1966). Internal standardizations of mobility variants were made for each new population sampled, by including TOD individuals of known genotype.

RESULTS

The esterase polymorphisms

Littorina littorea. Four major zones of esterase activity were observed in zymograms of individual *L. littorea*. Est 1 and Est 2 were completely monomorphic for the same allele in all the populations studied. Est 3 and Est 4, shown in

Figure 2, were both polymorphic, each locus having two alleles in the range of populations sampled. Homozygotes at either locus were characterized by a single major band, and heterozygotes displayed a three banded pattern characteristic of a dimeric enzyme in which subunit polypeptides associate at random.

Littorina obtusata. Three major zones of esterase activity were observed from single *L. obtusata* (Fig. 2). At the Est 1 locus three variant alleles were observed among the populations sampled, while the Est 3 and Est 4 polymorphisms consisted of two alleles each. Est 1 heterozygotes displayed two banded patterns, while heterozygotes at the Est 3 and Est 4 loci had the more typical three banded pattern. In all cases homozygotes yielded a single band.

Littorina saxatilis. A variable number of esterase zones were detected in individual homogenates of *L. saxatilis*. Because of the complex banding pattern in the cathodal region of the gel only the two anionic systems were evaluated. At the Est 3 locus in this species three alleles were found at high frequency, and at the Est 4 locus two common alleles were detected. Both gene-enzyme systems showed, in the homozygote, a single band; and in the heterozygote a three band pattern (Fig. 2).

In our buffer system the F allele at the Est 3 locus, and both the F and S alleles at the Est 4 locus, appeared to have indistinguishable mobilities in all three species. Similarly, the S allele at the Est 3 locus had identical mobilities in both *L. littorea* and *L. saxatilis*. The Est 3^S allele of *L. obtusata*, and the Est 3^{S'} *L. saxatilis* appeared species specific. These mobility identities, however, do not constitute final proof of their chemical identity.

Allele frequencies in natural populations

Littorina littorea. The distribution of allele frequencies in the 13 populations sampled is presented in Tables I and II. The predominant allele at the Est 3 locus is the F variant. The Est 3^S allele while common (present in 6 of the 13 populations) is maintained at low frequency (0.03 to 0.36) in those populations surveyed. At the Est 4 locus the S allele is fixed in 11 of 13 populations, and nearly so in two others (WHO and BAR).

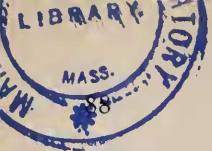
Littorina obtusata. Allele frequency determinations for the 14 populations of *L. obtusata* sampled are included in Tables I and II. At the Est 1 locus the F allele was found at relatively high frequency in the southern Cape Cod region (0.36 to 0.60), while in populations north of the Cape Cod Canal this allele occurred at lower frequencies (0.02 to 0.40). The most northern population, from PEI, was completely lacking this variant. The Est 1^S allele was observed in all the populations at frequencies ranging from 0.35 to 0.90. The PEI population was characterized by a unique allele, Est 1^{S'}, whose frequency was 0.57.

At the Est 3 locus both alleles were found at high frequency in all but the FAY population, where Est 3^S was fixed. No pattern of clinal variation could be discriminated. The Est 4 polymorphism was observed in 11 of the populations. In the northern species range the frequency of Est 4^F was low, but in several populations clustered around WHO the Est 4^F allele reached high values (0.44–0.52).

Littorina saxatilis. As was the case for *L. obtusata*, one of the polymorphic loci in *L. saxatilis* showed marked regional differentiation. In the southern Cape

TABLE I
Esterase phenotypes for Littorina litorea, L. obtusata, and L. saxatilis for the 15 localities sampled. Dashed lines indicate the absence of a species at that site. O refers to other genotypes; in addition, for L. obtusata, at PEL.*
Est 1 are: SS' = 9; SS' = 13.

Site	<i>L. obtusata</i>									<i>L. saxatilis</i>									<i>L. litorea</i>								
	Est 1			Est 3			Est 4			Est 3			Est 4			Est 3			Est 4			FF	FS	SS	FF	FS	SS
	FF	FS	SS	O*	FF	FS	SS	FF	FS	SS	FF	FS	SS	FF	FS	SS	FF	FS	SS	FF	FS	SS					
COT	2	16	2	0	0	7	12	4	9	7	—	—	—	—	—	—	13	1	1	0	0	25	—	—	—	—	—
MAV	1	8	5	0	2	7	5	0	0	14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
FAY	2	7	0	1	0	0	10	0	1	9	—	—	—	—	—	—	14	0	0	0	0	20	—	—	—	—	—
NOB	5	12	4	0	3	5	13	3	6	11	—	—	—	—	—	—	7	0	0	1	1	35	—	—	—	—	—
WHO	5	12	6	1	2	12	8	6	12	15	—	—	—	—	—	—	32	6	0	0	0	43	—	—	—	—	—
TOD	2	17	5	0	1	11	12	5	12	6	—	—	—	—	—	—	12	1	1	0	0	15	—	—	—	—	—
SIP	8	10	6	0	5	12	9	3	15	6	—	—	—	—	—	—	7	3	3	0	0	26	—	—	—	—	—
SIB	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	0	0	0	0	2	—	—	—	—	—
BAR	4	10	9	0	1	7	16	1	9	14	—	—	—	—	—	—	6	0	0	0	0	12	—	—	—	—	—
SAN	2	5	17	0	2	13	9	0	8	16	—	—	—	—	—	—	9	3	0	0	0	12	—	—	—	—	—
MAN	0	10	13	0	4	11	9	0	4	20	—	—	—	—	—	—	7	0	0	0	0	9	—	—	—	—	—
GLO	0	1	23	0	6	9	9	2	14	8	—	—	—	—	—	—	12	0	0	0	0	12	—	—	—	—	—
KIT	0	9	15	0	3	10	11	0	1	23	—	—	—	—	—	—	6	0	0	0	0	6	—	—	—	—	—
BHA	2	15	9	0	3	9	12	0	0	24	—	—	—	—	—	—	10	0	0	0	0	17	—	—	—	—	—
PEL+	0	0	4	1	1	6	21	0	0	27	—	—	—	—	—	—	14	3	0	0	0	22	—	—	—	—	—



Cod populations both Est 3^F and Est 3^S were found at high, and roughly similar frequencies (Tables I and II). Proceeding north from Cape Cod the frequency of the Est 3^F allele gradually decreased reaching a minimum value of 0.10 at GLO and KIT and BHA. This reduction was not accompanied by a coordinate increase in Est 3^S frequency, but rather by the appearance and gradual increase in frequency of a new variant, Est 3^{S'}. At PEI the frequency of Est 3^F is again high, 0.51, and the frequency of Est 3^{S'} low, 0.15. The Est 4 polymorphism was observed in 7 of the 12 populations sampled. The Est 4^F allele is generally at low frequency, in all populations, although at the SAN locale a value of 0.31 was recorded.

TABLE II

Esterase allele frequencies for Littorina littorea, L. obtusata and L. saxatilis in the 15 localities sampled. Dashed lines indicate the absence of a species at that site. O refers to other rare variants.*

Site	<i>L. obtusata</i>								<i>L. saxatilis</i>						<i>L. littorea</i>			
	Est 1				Est 3		Est 4		Est 3			Est 4			Est 3		Est 4	
	F	S	S'	O*	F	S	F	S	F	S	S'	F	S		F	S	F	S
COT	0.50	0.50	0	0	0.18	0.72	0.43	0.57	—	—	—	—	—		0.97	0.03	0	1.0
MAV	0.36	0.64	0	0	0.39	0.61	0	1.0	—	—	—	—	—		—	—	—	—
FAY	0.60	0.35	0	0.05	0	1.0	0.05	0.95	0.40	0.60	0	0.02	0.98		—	—	—	—
NOB	0.52	0.48	0	0	0.26	0.74	0.30	0.70	0.54	0.46	0	0.03	0.97		1.0	0	0	1.0
WHO	0.50	0.49	0	0.01	0.35	0.65	0.52	0.48	—	—	—	—	—		1.0	0	0.03	0.97
TOD	0.44	0.56	0	0	0.27	0.73	0.48	0.52	0.47	0.53	0	0.05	0.95		0.92	0.08	0	1.0
SIP	0.59	0.41	0	0	0.41	0.59	0.44	0.56	0.42	0.58	0	0	1.0		0.89	0.11	0	1.0
SIB	—	—	—	—	—	—	—	—	0.43	0.57	0	0	1.0		0.64	0.36	0	1.0
BAR	0.40	0.60	0	0	0.19	0.81	0.23	0.77	0.31	0.69	0	0	1.0		1.0	0	0.08	0.92
SAN	0.19	0.81	0	0	0.35	0.65	0.20	0.80	0.17	0.68	0.15	0.31	0.69		0.88	0.12	0	1.0
MAN	0.22	0.78	0	0	0.40	0.60	0.08	0.92	0.19	0.54	0.27	0.02	0.98		1.0	0	0	1.0
GLO	0.02	0.98	0	0	0.44	0.56	0.37	0.63	0.10	0.40	0.50	0	1.0		1.0	0	0	1.0
KIT	0.19	0.81	0	0	0.33	0.67	0.02	0.98	0.10	0.60	0.30	0	1.0		1.0	0	0	1.0
PEI	0	0.41	0.57	0.02	0.15	0.85	0	1.0	0.51	0.34	0.15	0.01	0.99		0.92	0.08	0	1.0
BHA	0.37	0.63	0	0	0.31	0.69	0	1.0	0.10	0.57	0.33	0.14	0.86		1.0	0	0	1.0

DISCUSSION

Our main objective in these studies has been to assess the effect of varying levels of larval dispersal on the genetic structure of three littorinid species. Although we have no breeding data for the enzyme systems studies the patterns of electrophoretic banding appears to indicate the presence of first order allelic variation at three of the esterase systems described. The major result is that in those species endowed with limited dispersal capabilities (*L. saxatilis* and *L. obtusata*) one can find both geographic differentiation with respect to allele frequencies, and, in certain cases, alleles unique to a specific geographic region. In *L. littorea* a gastropod with extensive dispersal capabilities, owing to its lengthy pelagic period, no evidence was found for either significant regional differentiation, or region specific alleles. While it would be of additional interest to measure and compare allele frequency variance in these species, this method breaks down for extremely small values of one allele frequency, and, of course, becomes absolutely useless if there is monomorphism. Thus the low frequency alleles of *L. littorea* would tend to generate lower variance values (the predicted result) simply because of the

low level of heterozygosity. Additional studies of polymorphic loci are needed, then, to eliminate this bias, and such studies are in progress.

Is the correlation between dispersal capability and the genetic differentiation of species unique to littorinids? Gooch, Smith and Knupp, 1972, have reported the results of a similar electrophoretic study for two gene-protein systems in 11 coastal populations of the prosobranch gastropod, *Nassarius obsoletus*, collected over a geographic range of 700 miles. This species like *L. littorea* has pelagic eggs and larvae, whose dispersal capabilities have been estimated to extend far over 250 miles, about one-fourth of its North American range. The results are unambiguous. For the diallelic LDH locus the range in frequencies of the major variant is 0.52–0.60, for the Pt locus, also diallelic, the frequency range of the major allele is 0.62–0.70. Thus the homogeneity of *Nassarius* populations is far more dramatic than even that of *L. littorea*, and indeed reflects the much broader dispersal range of *Nassarius*, compared to *L. littorea*.

The varying levels of genetic heterogeneity between populations may be interpreted in several ways. With limited dispersal (gene flow), one might argue, populations are afforded the opportunity to adapt genetically to the peculiar environmental conditions (both physical and biotic) in their locale. With local environmental conditions varying over the species range, one could anticipate an accompanying genetic differentiation. Alternatively, it is possible to suggest that isolation *per se* enhances the effect of random drift, serving effectively to promote genetic differentiation. Data on the distribution of gene frequencies cannot distinguish between these possibilities, for estimates of effective population size or actual dispersal rates have not been made in these species. It might be of interest, in distinguishing the effects of drift from selection, to examine many loci in these species and derive some estimate of the average individual heterozygosity. If random drift were effectively driving genes to fixation in species characterized by significant interpopulation heterogeneity then this should be reflected by low values of heterozygosity. Such a situation appears to be the case in the isolated Bogota population of *Drosophila pseudoobscura* (Prakash, Lewontin and Hubby, 1969), and in the marginal island populations of the field (mouse *Peromyscus polionotus* off the Florida coast (Selander, Smith, Yang, Johnson and Gentry, 1971). The selective model does not require this necessary condition.

In evolutionary terms the varying levels of larval dispersal in the three littorinid species may promote two additional effects. First, associated with the capability for extensive gene flow is the potential for widespread colonization. This is amply demonstrated by the recent and rapid appearance of *L. littorea* along the western Atlantic coast (Sumner *et al.*, 1911). The second, and closely related, effect associated with different levels of gene flow is that limited dispersal may promote genetic differentiation to the point of reproductive isolation. This appears to be true in gastropod molluscs where the greatest level of subspecies formation occurs in those forms showing the more restricted dispersal capabilities (Scheltema, 1971).

Dispersal rates, and the degree of gene flow between populations, are unknown for most species. For this reason, perhaps, these factors are often not considered explicitly in experimental studies of species variation. Nevertheless, it is clear

that for the littorinids, and probably most prosobranch gastropods, mechanisms of dispersal may play a fundamental role in establishing the genetic structure of the species, and have a large impact on its evolutionary potential.

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SUMMARY

Gene-enzyme variation was examined, electrophoretically, at three non-specific esterase loci in 15 sympatric populations of the prosobranch gastropods: *Littorina littorea* L. *obtusata*, and *L. saxatilis*. Gene frequencies, determined by assuming a correspondence between bands on gels and alleles, revealed marked differences between the species. These differences appear to be correlated with the mechanisms and capabilities for larval disposal in these species.

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